

Chromogenic *Bacillus Cereus* Selective Supplement

PRODUCT INFORMATION

P007-1MU - Polymyxin B Sulfate, Powder, 1MU

P007-10MU - Polymyxin B Sulfate, Powder, 10MU

P007-100MU - Polymyxin B Sulfate, Powder, 100MU

T011-5g - Trimethoprim, Powder, 5g

T011-25g - Trimethoprim, Powder, 25g

T011-100g - Trimethoprim, Powder, 100g

DESCRIPTION

Chromogenic *Bacillus cereus* agar and chromogenic *Bacillus cereus* selective supplement is a chromogenic medium for the isolation and differentiation of *Bacillus cereus* from food samples.

BACKGROUND

Polymyxin B is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium *Bacillus polymyxa*. Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the *Proteus* group.

Trimethoprim is a bacteriostatic antibiotic which belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors.

Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

Trimethoprim acts by interfering with the action of bacterial dihydrofolate reductase, inhibiting synthesis of tetrahydrofolic acid. Tetrahydrofolic acid is an essential precursor in the de novo synthesis of the intermediate Thymidine monophosphate (dTMP), precursor of DNA metabolite Thymidine triphosphate. Bacteria are unable to take up folic acid from the environment (i.e. the infection host) and are thus dependent on their own de novo synthesis. Inhibition of the enzyme starves the bacteria of nucleotides necessary for DNA replication

causing, in certain circumstances, cell lethality due to thymineless death.

APPLICATION IN CHROMOGENIC *BACILLUS CEREUS* AGAR

Bacillus cereus, a Gram-positive, aerobic, spore-forming rod-shaped bacterium, is widely distributed in nature. It is readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water and sediments. Therefore, it is not surprising to find the organism associated with virtually every raw agricultural commodity. The ability to form spores ensures survival through all stages of food processing short of retorting, and the organism is present in most raw materials used in food manufacture. Under normal circumstances, *B. cereus* is found at $<10^3$ cells per gram of food and does not cause any problems as the minimum level to cause illness is more than 10^5 cells per gram.

Bacillus cereus associated gastroenteritis results from the ingestion of two distinct toxins (emetic toxin and enterotoxin) produced during the vegetative stage of growth, in foods that have been poorly refrigerated following cooking. Two types of illness are caused by the two toxins. The diarrhoeal type of illness is caused by a large molecular weight protein or enterotoxin. Onset is usually within 6-15 hours of ingestion of contaminated food. The vomiting (emetic) type of illness is believed to be caused by a low molecular weight, heat-stable peptide and symptoms can start to occur within 0.5-6 hours of ingestion.

A wide variety of foods including meats, milk, vegetables, and fish have been associated with the diarrhoeal-type food poisoning. The vomiting-type outbreaks have generally been associated with rice products; however, other starchy foods, such as potato and pasta, and cheese products have also been implicated. Food mixtures such as sauces, puddings, soups, casseroles, pastries, and salads have frequently been incriminated in food poisoning outbreaks.

Brilliance *Bacillus cereus* agar incorporates the chromogenic substrate 5-bromo-4-chloro-3-indolyl- β -glucopyranoside, which is cleaved by the enzyme β -glucosidase present in *Bacillus cereus* resulting in the formation of blue/green colonies. Polymyxin B inhibits most Gram-negative organisms and some

Gram-positive organisms including some *Bacillus* other than *Bacillus cereus*. Trimethoprim, which is also added to the medium, blocks folic acid synthesis necessary for DNA production and is active against many Gram-positive bacteria including *Staphylococcus aureus*, *Enterococcus* spp. and some non-*cereus* *Bacillus* species. The combination of these two antibiotics has been shown to be more effective than the use of polymyxin B alone.

Because *Bacillus thuringiensis* is biochemically identical to *Bacillus cereus*, it will also grow as blue/green colonies on this medium. *Bacillus thuringiensis* is known primarily as an insect pathogen, but it has also been reported to have been linked to some human gastroenteritis outbreaks.

Content concentrations

Typical Formula*	mg/litre
Chromogenic <i>Bacillus cereus</i> agar	
Yeast extract	4
Peptone	10
Di-sodium hydrogen phosphate	2.52
Potassium di-hydrogen phosphate	0.28
Sodium pyruvate	10
Chromogenic mix	1.2
Agar	13
Final pH 7.2 ± 0.2 @ 25°C	
Chromogenic <i>Bacillus Cereus</i> Selective Supplement	
Polymyxin B	106,000 IU
Trimethoprim	10.0
* Adjusted as required to meet performance standards	

Table 1 typical formula for chromogenic *Bacillus cereus* agar and chromogenic *Bacillus cereus* selective supplement

METHOD

Preparation

Suspend appropriate amount of chromogenic *Bacillus cereus* agar in distilled water. Mix well and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C and aseptically add 1 vial of Brilliance bacillus cereus selective supplement. Mix well and pour into sterile Petri dishes.

Protocol

Please note that the following is only intended as a suggested method of use

1. Prepare a 10% dilution (w/v) of the food sample to be tested in peptone water

2. Homogenise the sample for 1 minute using an appropriate laboratory blender

3. Inoculate 0.1 ml volumes of 10-1, 10-2 and 10-3 dilutions of the homogenate on to the surface of Chromogenic *Bacillus cereus* agar plates

4. Incubate the plates at 37°C for 24 hours

5. Examine for typical colonies of *Bacillus cereus*

6. Confirm the presumptive identification of *Bacillus cereus* by a validated method, e.g. oxidase, Gram-stain

7. Report the results as the number of *Bacillus cereus* colonies per gram weight of the food sample.

Quality control

Positive control:

Bacillus cereus ATCC®10876: Good growth; blue/green colonies

Negative control:

Bacillus subtilis ATCC®6633: No growth

Escherichia coli ATCC®25922: No growth

REFERENCES

- Foodborne Pathogenic Microorganisms and Natural Toxins Handbook *Bacillus cereus* and other *Bacillus* spp. (2003) U.S. Food & Drug Administration (C.F.S.A.N)
- The Oxoid Manual (1998) 8th Edition. Oxoid, UK.
- Poster – Cloke, J. M., Ring, M., Campbell, S., Smith, E., and Stringer, J. Evaluation of a new Oxoid chromogenic medium for the Isolation of *Bacillus cereus* from foods. (2003) Oxoid & Burton's Foods, UK.
- Handbook of Culture Media for Food Microbiology (2003) Volume 37. Chapter 4. Media for *Bacillus* spp. and related genera relevant to foods. Edited by Corry, J. E. L., Curtis, G. D. W. and Baird, R. M. Publisher - Elsevier, Amsterdam.